PHYSIOLOGICAL STUDIES ON THE CORN DIPLODIAS

BY

WILLIAM ERNEST WILSON

B.S., Wheaton College, 1936
M.S., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
BOTANY IN THE GRADUATE SCHOOL OF
THE UNIVERSITY OF ILLINOIS, 1941

Urbana, Illinois
1941

PHYSIOLOGICAL STUDIES ON THE CORN DIPLODIAS

BY

WILLIAM ERNEST WILSON

B.S., Wheaton College, 1936
M.S., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
BOTANY IN THE GRADUATE SCHOOL OF
THE UNIVERSITY OF ILLINOIS, 1941

Urbana, Illinois
1941

ACKNOWLEDGMENT

Hearty thanks are due Professor Neil E. Stevens for the sustained interest which he showed throughout the entire course of these studies, and for his very many helpful suggestions and criticisms.

The writer is also indebted to Dr. Donald E. Wolf and to Mr. Carl Stevens of the Department of Chemistry for their valued assistance and cooperation.

PHYSIOLOGICAL STUDIES ON THE CORN DIPLODIAS

Introduction

The culture of fungi in synthetic nutrient solutions has attained importance in recent years as a method of studying their physiological processes and metabolic requirements. The part which the so-called "trace elements" play in plant nutrition and likewise the metabolic role of various organic growth factors have been studied rather extensively. A survey of the literature, however, reveals that fungi which have little or no importance as plant pathogens have been the subjects of the majority of these studies. It is therefore highly desirable that more of the economically significant pathogenic fungi be included in such investigations.

The studies reported in this abstract deal with two of the organisms which attack maize. Some work has been done already on the physiology of these two fungi, but the results reported, especially as regards their utilization of simple sugars, have been somewhat conflicting. Miss Kinsel reported[1] that *D. zeae*, a common cause of corn ear rot, grew vigorously in culture and was able to utilize both simple and complex carbohydrates under the conditions of her experiments. In contrast, *D. macrospora*, a related ear rot fungus which is restricted in its known range in the U. S. to the southeastern portion, showed less vigorous growth when cultured and grew only when supplied with di- or polysaccharides as carbohydrates. Stevens and Larsh[2] using 24 isolates of *D. macrospora* from the southeastern U. S. and Argentine found that the characteristic noted by Miss Kinsel was common to all these isolates

1. Kinsel, Katherine. Carbohydrate Utilization by the Corn Diplodias. Phytopathology 27: 1119-20. 1937.
2. Stevens, Neil E., and Howard W. Larsh. Carbohydrate Utilization by *Diplodia macrospora*. Ill. State Acad. Science Trans. 32: 82. 1939.

regardless of the source of nitrogen supplied. More recently it has been indicated by Margolin[3] that *D. macrospora* develops equally well when supplied with either sucrose or glucose, growth on either sugar being sparse unless biotin or a biotin-like growth factor is supplied.

STUDIES OF NUTRIENT MEDIA

The basic medium used in the culture work contained the following substances: $MgSO_4$ 0.25 g., KH_2PO_4 0.3 g., KNO_3 2.0 g., carbohydrate 30.0 g., and distilled water to make one liter. One check culture of *D. zeae* was used in each lot of modified medium which was tested for its ability to support a mycelial growth of *D. macrospora*. The fungi were grown at room temperature in 125 ml. Pyrex flasks containing 50 ml. of medium which had been autoclaved at 15 pounds for 20 minutes. In most instances 10 flasks of each medium were inoculated. Bits of dormant mycelium from old cultures which were brown and showing no signs of growth were used as inoculum.

Varied Sources of Inoculum. Inoculum taken from an old dormant culture of *D. macrospora* invariably gave no significant growth on the basic media containing only a hexose sugar as a source of carbohydrate. Experiment showed that a wisp of the mycelium from a vigorously growing oat culture developed an excellent growth when placed in a dextrose medium. Hyphae from corn meal agar plates inoculated with kernels of corn infected with *D. macrospora* also gave rise to a sparse mycelium in dextrose media.

Growth of D. macrospora on Media Staled by Other Fungi. When the mycelium is removed by filtration from dextrose media staled 3 or 4 weeks by *D. zeae* and the solution re-autoclaved, *D. macrospora* will then develop very well. The same characteristic obtains for media containing galactose, rhamnose, mannite, sorbose, levulose, and also glycerol.

3. Margolin, A. S. The Carbohydrate Requirements of *Diplodia macrospora*. Proc. W. Va. Acad. Science, 1940, 14 (Keyser), in W. Va. Univ. Bul. Ser. 41, no. 4-11. 1940.

Growth Promoting Substances. A powder obtained from corn grains and likewise from whole oats by steaming the grains and extracting the aqueous residue with alcohol exerted a definite stimulating effect upon the growth of *D. macrospora.*

Green corn stalks, stock molasses, sugar beets, and a dextrose solution staled by *D. zeae* also provided similar growth promoting substances when extracted with alcohol.

A biotin-like substance extracted from dung enabled *D. macrospora* to form a heavy mycelium on a sucrose medium purified with Norite. Commercial biotin concentrate produced mats of *D. macrospora* averaging 115.5 mg. when 0.02 gamma of biotin per 50 ml. flask of dextrose medium were added. "Difco" invertase solution also induced growth of the fungus in dextrose media.

Ineffective Growth Substances. Nicotinic acid, vitamin C, i-inositol, vitamin B_1, vitamin B_6, and a combination of vitamins C and B_1 induced no growth whatsoever of *D. macrospora* on dextrose media in the concentrations tested.

THE PROBABLE IDENTITY OF THE GROWTH FACTOR PRODUCED BY DIPLODIA ZEAE

Since it was known that a commercial preparation containing biotin enabled *D. macrospora* to utilize dextrose, an effort was made to determine whether the substance excreted by *D. zeae* may be identical with biotin. A concentrate of dextrose media staled by *D. zeae* also supported a good mycelial formation by *D. macrospora* when added in small quantities to unstaled dextrose media. For this reason the concentrate was subjected to a number of the treatments by which biotin, coenzyme R, and vitamin H, three growth factors now considered to be identical, are tested.

The growth substance present in staled media was found to be thermostable, soluble in water and alcohol, adsorbed by char, and scarcely soluble in ether, petroleum ether, or chloroform. It was also resistant to the action of strong acids and an alkali, dialyzable, and inactivated by nitrous acid; but re-

mained active after refluxing with acidulated methanol. Cooked egg albumen did not affect the activity of the growth substance but raw egg white inactivated it completely for *D. macrospora*. The growth substance also stimulated the growth of *Saccharomyces cerevisiae*, a yeast believed to require biotin.

GROWTH FACTOR PRESENT IN CERTAIN CARBOHYDRATES

The fact that *D. macrospora* will grow readily on simple sugars if supplied with suitable growth substances suggested that the results reported by Miss Kinsel and by Stevens and Larsh might be due to impurities present in the complex carbohydrates which are removed from the monosaccharides in the course of further processing. It also seemed reasonable to believe that very pure di- or polysaccharides might also be lacking in the impurities needed for the growth of the fungus.

Studies on the Preparation of Dextrose. A number of samples of hydrolyzed starch from various stages of the processing by which starch is converted to dextrose were obtained from the Corn Products Refining Co. and tested for their ability to support growth of the two Diplodias. The results obtained are listed in the following table.

TABLE 1

Growth record of *D. macrospora* and *D. zeae* cultured for 7 weeks at room temperatures.

Sample	*D. macrospora* No. of Flasks	*D. macrospora* Av. Net Wt.	*D. zeae* (1 Flask)
Starch hydrolysate neutralized	9	109.4 mg.	Good
Liquor passed over char once	9	240.0	Good
Liquor ready for crystallizer	8	93.7	Good
Hydrol	9	294.4	Good
Dextrose	9	Negligible	Fair
Dextrose plus 10 g. of Hydrol per liter		197.2	Fair
Dextrose plus 10 g. of fatty centrifugate per liter		Negligible	Poor
Special Hydrol (40 g. per l.)		97.0	Fair
Special Hydrol (60 g. per l.)		97.1	Fair

Studies on Inulin and Maltose. *D. macrospora* made only a scant growth when supplied with two samples of inulin, but varied widely in its development on maltose. The growth obtained on a brown, technical grade of maltose exceeded that obtained on a C.P. grade by over 1400%. Passage of the technical grade maltose through animal char effectually prevented any growth of *D. macrospora.*

Studies on Sucrose. Growth also varied on sucrose samples from five sources which were tested for their ability to support development of *D. macrospora.* The comparative results are given in the following table.

TABLE 2

Growth record of *D. macrospora* and *D. zeae* cultured for 4 weeks at room temperatures on various samples of sucrose.

Brand of Sucrose	*D. macrospora* (Av. of 9 Flasks)	*D. zeae* (1 Flask Only)
Bakers "Analyzed" C.P.	41.1 mg.	Satisfactory
Coleman & Bell C.P. "Saccharose"	90.0	Satisfactory
C & H "Pure Cane" from 2 lb. box	48.1	Satisfactory
Domino Cube	27.8	Satisfactory
C & W Beet Sugar from 10 lb. bag	Very sparse	Satisfactory

The results obtained from the beet sugar were surprising because it was found that sugar beet roots yielded a growth factor which enabled *D. macrospora* to utilize both beet sugar and dextrose. On the other hand, some investigators have found that beet sugar molasses contains appreciably less biotin than does cane sugar molasses. *D. macrospora* consistently failed to make significant growth in our experiments when supplied with Domino Cube sugar. This is apparently due to its very high purity (99.95%) in contrast to the usual run of cane sugar which has a purity of about 99.5%.

SUMMARY

The source of inoculum of *D. macrospora* was found to be correlated with the ability of this fungus to grow on simple sugars supplied in the basic nutrient medium.

A substance which enabled *D. macrospora* to utilize simple sugars was obtained from corn, oats, sugar beets, molasses, hyphae of *D. macrospora* from vigorously growing cultures, and a dextrose medium staled by *D. zeae*. An invertase solution and a commercial biotin concentrate were also effective in promoting growth.

The growth substance elaborated by *D. zeae* and present

in a concentrate of dextrose media staled by this fungus was shown to have many of the properties of biotin and is believed to be biotin or a closely related compound.

Tests made on samples from various stages of the process by which starch is converted to dextrose indicated that the growth factor present in starch is not present in dextrose in significant amounts, but remains in the mother liquor which is removed from the dextrose after crystallization.

The growth of *D. macrospora* was definitely influenced by the source of the maltose and sucrose supplied in the culture media.

The results obtained indicate that *D. macrospora* can utilize complex carbohydrates in many instances only because they contain as an impurity a growth factor required by this fungus. This growth factor apparently is usually removed from simple sugars during the manufacturing processes.

VITA

William Ernest Wilson was born at Sparta, Illinois on June 7, 1907. He received his elementary and secondary education in the Sparta public schools, graduating from Sparta Township High School in June, 1924. The year following graduation he took post graduate work in the high school.

Beginning in the autumn of 1925 he taught three terms in the rural schools of Randolph County, Illinois. Subsequent to this he became associated with the Rockford Electric Co. of Rockford, Illinois and was employed in the line department of that organization until the summer of 1932.

He entered Wheaton College, Wheaton, Illinois in September, 1932 and was graduated with honor in June, 1936 with the degree of Bachelor of Science. During his Junior and Senior years he held an undergraduate assistantship in botany. After graduation he spent one year as full time graduate assistant in biology at Wheaton.

The University of Illinois awarded him a graduate assistantship in botany in 1937 which he held during four winter terms and two summer sessions. At the end of his second year of graduate work he was granted the degree of Master of Science in botany.

He was married to Miss Enid W. Dresser of Wheaton, Illinois in June, 1940 and has made his home in Urbana, Illinois since that time.

He is a member of Sigma Xi and The American Phytopathological Society.